DETERMINATIONS OF BLOOD PYRUVIC ACID, LACTIC ACID, HEMOGLOBIN, AND ERYTHROCYTES IN VARIOUS PATHOLOGIES

BY

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CHAPTER I

INTRODUCTION

The knowledge that pyruvic acid is a highly reactive biochemical intermediary of carbohydrate, protein and fat metabolism has stimulated this research. It is believed that a diseased organism is in a state of considerable stress and strain in which the need for large quantities of energy are required. This energy is supplied by the oxidation of protein, fat and carbohydrate, particularly the latter. Thus, an increase in the energy requirements of the body should, in the opinion of the investigator, be met by an increase in the oxidation of available sources of energy, with an accompanying increase in the concentration of blood pyruvate. It was further considered that the formulation of a diagnostic aid of clinical importance could be developed by noting the concentration of this biochemical substance in these different pathological states. In taking this view, the scope of this problem was not to establish a cause of any deviation from normal that may have been present in these various diseases but to determine if any abnormal quantities of pyruvic acid did prevail.

In searching the literature, it was found that very little work has been published in which pyruvic acid determinations were made in various pathological conditions. The reports which have been published in the literature are conflicting and scanty (1, 4, 7, 14).

Determinations of lactic acid were carried out in conjunction with pyruvic acid because of their direct relationship with each other in body metabolism. Hemoglobin and erythrocyte counts were performed because the high reactivity of pyruvic acid depends greatly upon the oxygen supply (13). Leucocyte counts were made to complete the blood picture.

CHAPTER II

LITERATURE SURVEY

Carbohydrate metabolism was not clearly understood until the year 1897 when Hans and Buchner made the key discovery of the use of cell free yeast extract to aid in the isolation of carbohydrate intermediaries. This was soon followed by the isolation of hexose phosphate by Harden and Young in 1905 (3). The discovery of this first intermediate compound of carbohydrate metabolism set a score of researchers working on the problem of finding carbohydrate intermediaries. Baldwin (3) has stated that it is impossible to give an accurate historical account of subsequent work on fermentation and the isolation of intermediates of carbohydrate metabolism. A number of mistakes were made and rectified by researchers all over the world, many being made simultaneously.

Dakin and Dudley in 1913 were the first to describe a procedure for the determination of pyruvic acid in blood. Smedley and MacLean (15) reported a method in the same year, but it was inadequate for clinical purposes. Dakin and Dudley were also the first to use nitrophenylhydrazine in their method. These authors noted that pyruvic acid

hydrozone could be separated from other hydrozones by treatment with 10 percent sodium carbonate solution. Further treatment with sodium hydroxide produced a red color in direct proportion to the amount of pyruvate present. These principles have since been applied by Lu (14) and Beuding and Wortis (5) in their modifications of the analysis of pyruvic acid.

Lu's method employs the basic principles of Dakin and Dudley, and represents a great advancement in technique by the use of smaller quantities of blood. She did, however, retain three extractions with ethyl acetate and three with sodium carbonate. Friedman and Haugen (8) eliminated these six extractions without loss of accuracy and specificity. They used one complete extraction with ethyl acetate, and one with sodium carbonate solution. When the procedure is carried out in this manner, the hydrozones of glyoxals and aldehydes are extracted only slightly, while the hydrozone of pyruvic acid is extracted almost quantitatively. The hydrozones of glyoxals and aldehydes tend to form some salts with the 10 percent sodium carbonate solution, thus their interfering effects are minimized by reducing the number of extractions.

CHAPTER III

PROCEDURES

The Lumetron Photoelectric Colorimeter was used for the pyruvic acid and lactic acid determinations. The method for pyruvic acid was that of Friedman and Haugen (11), briefly outlined above.

A standardization curve was constructed by using redistilled C.P. Eastman pyruvic acid, collecting that portion that distilled between 64 to 67 degrees centigrade at 13 mm. of mercury in vacuum, using a water pump for the vacuum. Ten ml. of the middle portion of the distillate were placed into a tared 50 ml. beaker and the contents weighed. The difference in weight, 14.018 Gm. in a liter or 14.018 mg./ml., was taken as the standard.

The pyruvic acid was transferred quantitatively to a liter volumetric flask and diluted to volume. Two diluted standard solutions were then prepared from this stock solution.

1. Solution A.--Ten ml. of the standard stock solution were placed in a 200 ml. volumetric flask and diluted to volume, giving a concentration of 0.7 mg./ml.

2. Solution B.--Ten ml. of solution A were placed in a 100 ml. volumetric flask and diluted to volume, giving a concentration of 0.07 mg./ml.

The following amounts of the standard solutions A and B were transferred to 100 ml. volumetric flasks and diluted to volume.

Amount Used	mg./100 ml. of Blood
1 ml. of B	0.07
2 ml. of B	0.14
4 ml. of B	0.56
2 ml. of A	1.4
4 ml. of A	2.8
6 ml. of A	4.2
8 ml. of A	5.6
10 ml. of A	7.0

The C.P. ethyl acetate used was dehydrated and purified by extracting three times with 0.1 its volume of saturated solution of calcium chloride. Two hundred grams of anhydrous sodium sulfate were added for each liter of ethyl acetate, and allowed to stand several hours with frequent shaking. It was then distilled, and the portion coming over at 73 to 78 degrees centigrade was collected and used.

The Lumetron Photocolorimeter was standardized setting the galvanometer at 100 percent transmission and using filter 520.

Method Used for the Analysis

Five ml. of each of the pyruvic acid standard dilutions were placed in a test tube and 25 ml. of 10 percent trichloroacetic acid (freshly prepared) were added to each

tube with thorough mixing. Three ml. of this mixture were then transferred to a test tube containing 1 ml. of 0.1 percent 2,4-dinitrophenylhydrazine in 2N hydrochloric acid and allowed to stand ten minutes. Eight ml. of ethyl acetate were then added to the test tube and mixed by passing a stream of air through the mixture for two minutes. Six ml. of 10 percent sodium carbonate were then added to the contents of the tube and again mixed by passing a stream of air through the mixture for two minutes. Five ml. of the aqueous phase were pipetted into a Lumetron absorption tube, and 5 ml. of 1.5N sodium hydroxide were added to the contents and allowed to stand ten minutes before reading in the Lumetron Photoelectric Colorimeter.

The procedure for the unknown bloods was conducted as described above using 2 ml. of oxalated blood which was deproteinated with 10 ml. of 10 percent trichloroacetic acid (freshly prepared). This was then centrifuged and 2 ml. of the supernatant liquid was used for the analysis.

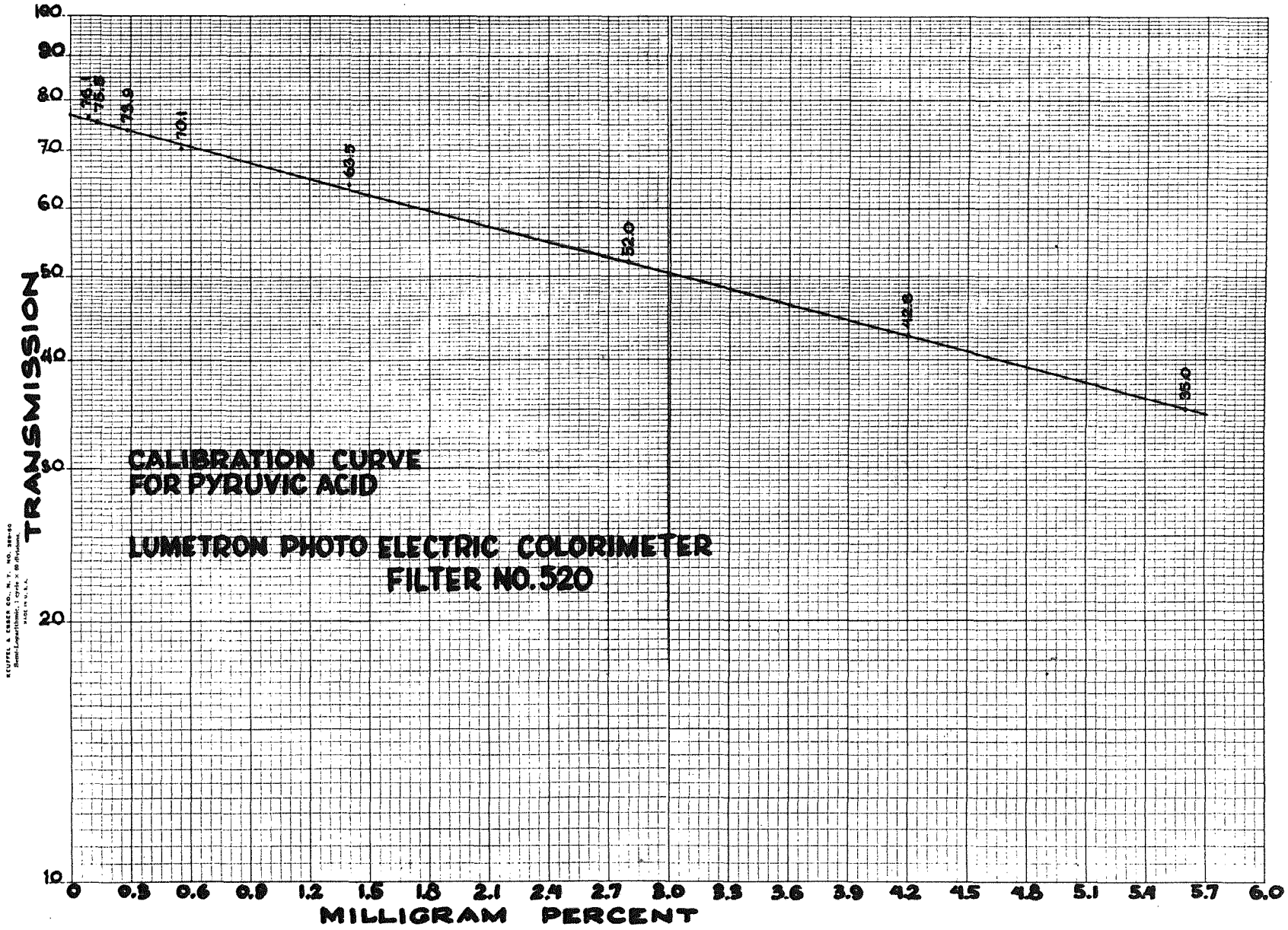
Lactic Acid Procedure

One ml. of oxalated blood and 6 ml. of water were placed into a test tube. A control containing 7 ml. of water was run simultaneously with each group of unknown blood samples. One ml. of 6 percent metaphosphoric acid was added, mixed, and centrifuged. Four ml. of the clear supernatant liquid were transferred by pipette to another

centrifuge tube to which was added 1 ml. of 15 percent copper sulfate solution and about 1 Gm. of calcium hydroxide. These tubes were stoppered and placed in the refrigerator for thirty minutes. They were then centrifuged and the supernatant liquid decanted and centrifuged again. One ml. of this clear liquid was placed in a test tube immersed in ice water, and 3 ml. of concentrated sulfuric acid added while stirring. One-tenth ml. of 20 percent alcoholic solution of hydroquinone was then added and the contents mixed. The solution was then placed in a boiling water bath for twenty minutes, then cooled in ice water for two minutes. Five ml. of water were then added, stirring until well mixed. The tubes were then allowed to stand until all gas bubbles had escaped, and the solution then transferred to a Lumetron absorption tube. Percent transmission of the unknown was read against a zero blank solution setting the galvanometer at 100 percent transmission using filter 420. The milligram percent of lactic acid was then found by reading a precalibrated graph.

Hemoglobin determinations were conducted according to the Leitz Photoelectric Colorimeter Hand Book using the Leitz Photoelectric Colorimeter.

Erythrocyte and leukocyte counts were made according to an accepted clinical method using the Spencer Bright Line Hemocytometer.


CALIBRATION CURVE
FOR PYRUVIC ACID
LUMETRON PHOTO ELECTRIC COLORIMETER
FILTER NO. 520
TRANSMISSION
MILLIGRAM PERCENT
100
90
80
70
60
50
40
30
20
10
0
0.3
0.6
0.9
1.2
1.5
1.8
2.1
2.4
2.7
3.0
3.3
3.6
3.9
4.2
4.5
4.8
5.1
5.4
5.7
6.0
76.1
75.8
73.9
70.1
63.5
52.0
42.0
35.0

CHAPTER IV

RESULTS

Blood was obtained from eighty-nine fasting hospitalized patients of ages ranging from twenty-five to eighty-seven years. The results are presented in tabular form according to the disease diagnosed by the attending physician. Normal values chosen for hemoglobin and erythrocyte counts for both men and women were 15 Gm./100 ml. of blood and 4,500,000 to 5,500,000 per cu. mm., respectively. Normal values for pyruvic acid were 0.6 to 1.2 mg./100 ml. of blood (11). The lactic acid normal was taken as 5 mg. to 20 mg./100 ml. of blood (11).

TABLE 1

RESULTS OF THE DETERMINATIONS OF PYRUVIC ACID, LACTIC ACID, HEMOGLOBIN, ERYTHROCYTE, AND LEUKOCYTE COUNTS IN PATIENTS WITH NEO-PLASTIC DISEASES

Normal Values				
15 Gm./100 ml.	4,500,000 to 5,500,000 cu.mm.	5,000 to 10,000 cu.mm.	0.6 to 1.2 mg./100 ml.	5 to 20 mg./100 ml.
Hemoglobin	R.B.C. Count	W.B.C. Count	Pyruvic Acid	Lactic Acid
7.4	2,690,000	17,900	0.7	*
9.4	3,910,000	6,900	0.55	*
11.0	3,960,000	13,500	0.43	*
11.0	5,590,000	18,859	0.0	. .
13.9	5,910,000	7,950	0.0	*
12.8	6,320,000	8,499	0.15	*
12.6	4,960,000	7,450	0.31	*
12.6	4,210,000	9,050	0.0	14.0
12.8	4,070,000	6,850	0.0	9.5
11.3	4,210,000	5,650	0.0	18.5
7.05	3,310,000	7,700	0.0	13.4
10.65	4,450,000	7,650	0.0	15.9
9.0	2,980,000	7,850	0.55	12.2
5.6	2,290,000	12,200	0.0	*
13.9	5,150,000	9,500	0.0	10.0
7.4	*	*	0.55	*
12.6	*	*	0.0	*

*Results not obtained.

TABLE 2

RESULTS OF THE DETERMINATIONS OF PYRUVIC ACID, LACTIC ACID, HEMOGLOBIN, ERYTHROCYTE, AND LEUKOCYTE COUNTS IN PATIENTS WITH CARDIAC DISEASES

Normal Values				
15 Gm./100 ml.	4,500,000 to 5,500,000 cu.mm.	5,000 to 10,000 cu.mm.	0.6 to 1.2 mg./100 ml.	5 to 20 mg./100 ml.
Hemoglobin	R.B.C. Count	W.B.C. Count	Pyruvic Acid	Lactic Acid
15	*	*	0.31	*
9.3	*	*	0.31	*
13	4,750,000	11,050	0.0	*
15.3	4,380,000	11,600	0.15	*
9.15	*	*	0.43	*
15.8	*	*	0.0	*
13.9	*	*	0.0	*
15.3	*	*	0.0	*
13.0	*	*	0.55	*
17.6	6,860,000	11,250	0.5	*
11.0	3,710,000	9,100	0.0	*
*	6,040,000	12,450	0.0	*
15.8	7,320,000	10,850	0.0	*
15.3	4,910,000	7,650	0.0	*
13.0	4,950,000	12,000	0.0	*
13.65	6,020,000	8,600	0.43	*
14.1	4,820,000	13,450	0.31	*
12	5,050,000	9,800	0.85	*
12.35	4,250,000	4,800	0.0	*
15.3	4,810,000	17,900	0.43	*

*Results not obtained.

TABLE 2--Continued

Normal Values				
15 Gm./100 ml.	4,500,000 to 5,500,000 cu.mm.	5,000 to 10,000 cu.mm.	0.6 to 1.2 mg./100 ml.	5 to 20 mg./100 ml.
Hemoglobin	R.B.C. Count	W.B.C. Count	Pyruvic Acid	Lactic Acid
14.8	*	*	0.0	*
12.5	5,990,000	10,050	0.0	*
11.8	5,070,000	10,800	0.0	10.6
11.8	3,820,000	8,950	0.0	11.2
14.55	6,020,000	5,950	0.0	15.2
10.65	3,990,000	14,650	0.0	*
13.0	3,890,000	8,100	0.15	7

TABLE 3

RESULTS OF THE DETERMINATIONS OF PYRUVIC ACID, LACTIC ACID, HEMOGLOBIN, ERYTHROCYTE, AND LEUKOCYTE COUNTS IN PATIENTS WITH DIABETES MELLITUS

Normal Values				
15 Gm./100 ml.	4,500,000 to 5,500,000 cu.mm.	5,000 to 10,000 cu.mm.	0.6 to 1.2 mg./100 ml.	5 to 20 mg./100 ml.
Hemoglobin	R.B.C. Count	W.B.C. Count	Pyruvic Acid	Lactic Acid
*	5,150,000	7,250	0.0	*
*	5,440,000	9,950	0.0	*
14.8	*	*	0.0	*
11.6	4,070,000	9,050	0.0	*
13.9	5,220,000	10,000	0.0	14.7
14.3	5,190,000	8,150	0.0	11.6
14.1	5,610,000	7,800	0.0	11.6
14.4	3,340,000	11,750	0.0	10.6
12.47	4,690,000	8,200	0.0	15.2
8.8	2,740,000	9,000	0.0	10.6

*Results not obtained.

TABLE 4

RESULTS OF THE DETERMINATIONS OF PYRUVIC ACID, LACTIC ACID, HEMOGLOBIN, ERYTHROCYTE, AND LEUKOCYTE COUNTS IN PATIENTS WITH DEGENERATIVE DISEASES

Normal Values	15 Gm./100 ml.	4,500,000 to 5,500,000 cu.mm.	5,000 to 10,000 cu.mm.	0.6 to 1.2 mg./100 ml.	5 to 20 mg./100 ml.
Prenicious Anemia.....	5.25	1,620,000	3,850	0.15	*
Prenicious Anemia.....	7.05	2,610,000	10,550	0.0	*
Anemia (undifferentiated).	*	3,160,000	4,800	0.0	14.0
Uremia.....	10.4	4,570,000	11,750	0.0	*
Uremia.....	9.5	3,590,000	15,000	0.0	18.5
Uremia and Anemia.....	6.92	1,920,000	9,350	0.0	17.2
Cirrhosis of Liver.....	11.0	3,330,000	8,150	0.0	13.4
Cerebrovascular Accident.....	*	*	*	0.31	10.6
Cerebrovascular Accident.....	14.55	5,180,000	6,850	0.0	11.0
Common Duct Stone.....	14.3	5,420,000	9,200	0.0	10.6
Rheumatoid Arthritis....	10.65	4,210,000	10,000	0.0	12.8

*Results not obtained.

TABLE 5

RESULTS OF THE DETERMINATIONS OF PYRUVIC ACID, LACTIC ACID, HEMOGLOBIN, ERYTHROCYTE, AND LEUKOCYTE COUNTS IN PATIENTS WITH INFECTIOUS DISEASES

Normal Values	15 Gm./100 ml.	4,500,000 to 5,500,000 cu.mm.	5,000 to 10,000 cu.mm.	0.6 to 1.2 mg./100 ml.	5 to 20 mg./100 ml.
Pneumonia.....	12.35	4,110,000	12,350	0.0	*
Pneumonia.....	11.5	4,690,000	6,400	0.0	16.5
Pneumonia.....	13.4	3,960,000	14,200	0.0	9.0
Pneumonia.....	7.25	3,120,000	8,850	0.31	17.8
Pneumonia.....	13.4	4,290,000	*	1.85	14.7
Pneumonia.....	13.4	4,710,000	5,900	0.0	17.8
Pneumonia.....	8.05	*	*	0.0	*
Pneumonia.....	13.9	*	*	0.0	11.6
Hepatitis.....	15.8	5,710,000	9,050	1.4	*
Hepatitis.....	13.0	4,240,000	4,650	0.43	15.0
Hepatitis and Pregnancy.....	9.15	5,450,000	7,900	0.0	14.7
Pyelonephritis..	13.4	4,440,000	11,850	0.0	12.8
Dermatophytosis.	14.5	4,440,000	8,800	0.0	11.6
Luetic Heart....	11.35	4,280,000	8,100	0.0	18.5
Pulmonary Emphysema.....	11.8	4,120,000	9,750	0.3	11.6
Pulmonary Emphysema.....	10.65	3,460,000	15,850	0.43	17.8
Pulmonary Tuberculous...	8.2	4,740,000	8,450	0.0	17.3

*Results not obtained.

TABLE 6

RESULTS OF THE DETERMINATIONS OF PYRUVIC ACID, LACTIC ACID, HEMOGLOBIN, ERYTHROCYTE, AND LEUKOCYTE COUNTS IN PATIENTS WITH MISCELLANEOUS DISEASES

Normal Values	15 Gm./100 ml.	4,500,000 to 5,500,000 cu.mm.	5,000 to 10,000 cu.mm.	0.6 to 1.2 mg./100 ml.	5 to 20 mg./100 ml.
Hysterectomy...	10.65	*	*	0.0	*
Asthma.....	*	*	*	0.85	12.8
Asthma.....	13.65	5,220,000	9,300	0.15	15.9
Pregnancy.....	12.35	4,450,000	14,200	0.0	11.2
Pregnancy.....	12.17	3,790,000	5,200	0.0	9.5
Common Duct Stone.....	14.3	5,420,000	9,200	0.0	10.6
Common Duct Stone.....	11.0	3,360,000	5,750	0.0	13.4

*Results not obtained.

CHAPTER V

DISCUSSION

The pyruvic acid concentration in patients with various pathologies are presented in Tables 1, 2, 3, 4, 5, and 6. The majority of cases studied had a 0 percent pyruvic acid or an insignificant amount. There were a few isolated cases which were within the normal range, but they were believed to be of no practical value. The hemoglobin concentration in the majority of cases studied was below the set standard. Thirty of the total number of patients studied were found to have normal, or above, erythrocyte counts. Lactic acid concentration in all patients in this study fell within the normal range.

The expectation of some correlation between the tests performed was not realized. It was particularly disappointing when no relationship was found between the hemoglobin and pyruvic acid concentration. It is the opinion of this investigator that the finding of normal concentrations of lactic acid is indicative of the fact that the pyruvic acid was not being converted immediately to lactic acid.

The expectation of obtaining high pyruvic acid levels was backed by the reports of Amatuzio, et al. (1, 2), Goldsmith (9), Earl, et al. (6), and Havel, et al. (10).

In diabetes mellitus, Bueding (5) and Hill (12) obtained normal fasting pyruvic acid levels with an increase after the ingestion of glucose and the administration of insulin. These reports, however, were contrary to the findings of Amatuzio, et al. (1), wherein they found no increase of pyruvic acid after the administration of insulin, but obtained normal fasting pyruvic acid levels. These authors also recorded increased pyruvic acid levels in liver coma. Liver coma was not studied in the course of this research, but the results of two cases of hepatitis were recorded. One of these patients had a pyruvic acid concentration of 1.4 mg. and a hemoglobin concentration of 15.8 Gm. The other had a 0.43 mg. percent of pyruvic acid and a hemoglobin concentration of 13.0 Gm., thus indicating the presence of pyruvic acid but not in increased quantities.

Friedman and Haugen in 1942 (7) reported that the use of anti-coagulants and an anti-enzyme would bring about an immediate loss of pyruvic acid. This is, however, contradictory to the results obtained by Goldsmith (9), Bueding and Wortis (4, 5), and Earl, et al. (5), wherein they obtained high levels of pyruvic acid in their experiments when using an anti-coagulant or anti-enzyme, or both.

It is the opinion of this investigator that due to the conflicting results that have been reported in the literature, and the negative results obtained in this experiment, more basic research should be conducted on this subject.

CHAPTER VI

SUMMARY

Determinations of blood pyruvic acid, lactic acid, hemoglobin, and erythrocyte counts were performed on eighty-nine fasting hospitalized patients with various pathologies.

The methods used for the determinations are described in detail, and the results obtained are presented in separate tables according to the general classification of the disease as diagnosed by the attending physician.

The results obtained and recorded for pyruvic acid were 0 percent, or an insignificant amount. The hemoglobin and erythrocyte counts, according to the standards of this experiment, were subnormal in over 50 percent of the cases studied. Lactic acid concentrations were found to be within the normal range in all patients included in this investigation.

It is the opinion of this author that more basic research on the determination of pyruvic acid in the blood should be conducted before an interpretation of clinical significance can be established.

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